Restoration of American shad and hickory shad in Maryland's Chesapeake Bay

2001 Final Progress Report

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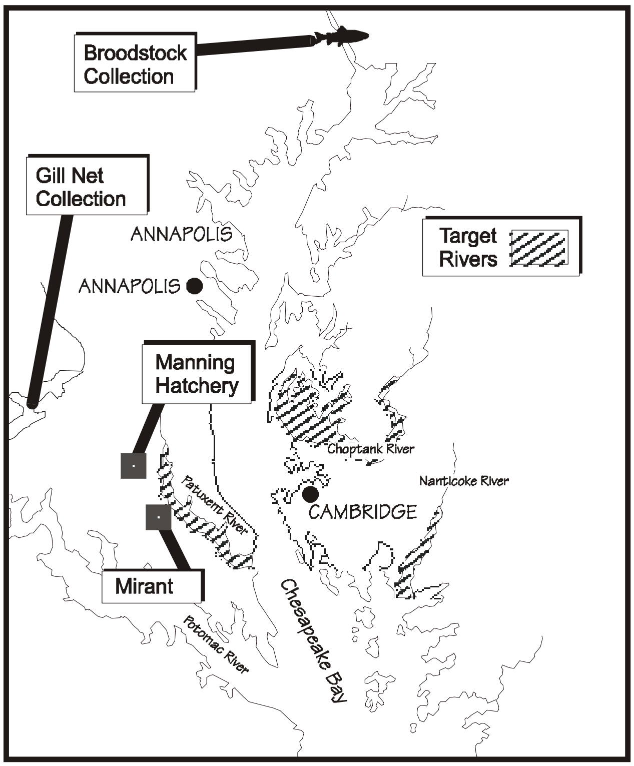
Sub-project 1.

Produce, mark and stock cultured American shad and hickory shad (Alosids) in Choptank River, Nanticoke River and Patuxent River.

Natural spawn production, marking and culture of hickory shad (*Alosa mediocris*) was conducted. Larvae were marked and stocked in the Choptank River, Marshyhope Creek and Patuxent River. Early juveniles were stocked in the Patuxent River and Choptank River. These fish were stocked as larvae in hatchery ponds and stocked into the rivers at approximately 30 days age. Late juvenile hickory shad were stocked in the Choptank River and Patuxent River. These fish were raised to approximately 80 days age in hatchery ponds and implanted with numerical coded wire tags (Northwest Marine Technologies, Bellvue, WA) before stocking in the rivers. American shad (*Alosa sapidissima*) larvae were produced, marked and cultured and stocked in the Patuxent River (Figure 1). American shad were also stocked as early juveniles and late juveniles in the Choptank River and Patuxent River. American shad production was lower than anticipated due to poor response to artificial hormone stimulation and low numbers of available brood for strip spawning.

Figure 1. *2001 target tributaries, brood collection and culture sites*



Materials and Methods

Hickory shad and American shad were produced using tank spawn culture methods developed by the project. Declining production of American shad over the last several years has dictated that an additional source of larvae be developed to supplement tank spawning operations. The decision was made to collect running ripe fish on the spawning grounds and manually strip eggs and milt from mature brood fish. A commercial fisherman was contracted to fish gill nets on the Potomac River at Marshall Hall (Figure 1). Nets were set near sunset on slack tides. DNR staff collected ripe fish from the nets, manually stripped eggs and milt through abdominal pressure and fertilized the eggs using the dry method (Howey 1985). Eggs were then allowed to water harden in river water. Water hardened eggs were packed in foam fish boxes and transferred to Manning Hatchery (Brandywine, Maryland) for culture.

Alosa brood stock for tank spawning operations was collected from the Susquehanna River. This Chesapeake Bay tributary has shown a spawning run resurgence in recent years. Hickory shad adults were collected by hook and line either immediately downstream of Deer Creek or at Shure’s Landing at the base of Conowingo Dam (Figure 1). Collection sites were chosen based upon susceptibility to capture. Angling gear consisted of light spinning tackle, small gold or silver spoons and shad darts of various colors. Recreational fishermen participated in collections. American shad brood stock was obtained from the fish lift operated by Susquehanna Electric Company at Conowingo Dam (Table 1).

Table 1. *2001 brood stock collection data.*

Date	Species	Females	Males
April 05	Hickory shad	46	77
April 09	Hickory shad	16	47
April 12	Hickory shad	5	44
April 16	Hickory shad	92	51
April 17	Hickory shad	42	24
April 18	Hickory shad	60	49
April 19	Hickory shad	49	29
April 23	Hickory shad	49	53
April 24	Hickory shad	54	21
May 02	American shad	64	142
May 14	American shad	73	110
May 21	American shad	62	42
May 31	American shad	47	37
June 06	American shad	60	50

Injections of synthetic analogs of gonadotropin-releasing hormone (GnRHa) stimulate pituitary release of endogenous gonadotropin, which induces gonadal maturation, ovulation and spawning (Mylonas et. al. 1995). A compassionate exemption from an Investigational New Animal Drug Permit (INAD #9222) was obtained from the U.S. Food and Drug Administration, which allows the experimental use of this drug. Current data indicates that doses of 25 to 50 micrograms per kilogram of body weight are suitable. LHRHa pellets for hickory shad were manufactured in the laboratory at Manning Hatchery using techniques developed by Lee et al. (1986). Powdered LHRHa (1.0 mg) was mixed with alcohol (0.5 ml), cholesterol (380 mg) and cocoa butter (20 mg). The mixture was dried at 37ºC for one hour. A form was constructed by drilling 4 mm holes in 6 mm Plexiglas. The paste was hammered into the Plexiglas form. After compaction the pellets were popped out of the Plexiglas mold and stored in cell trays. Cell trays were labeled and frozen in plastic bags with Dri-Rite desiccant. Implants for American shad were obtained commercially (Veripharm International, LLC., Richmond, Maine). Implants were stored in the hatchery freezer and transported to the field in coolers with ice packs.

Several controlled experiments were conducted in order to determine the ideal LHRHa implant concentrations needed for successful American shad tank spawning. American shad were either implanted with different concentration implants, sham implants or held as untreated controls. Egg volume and percent viable eggs were recorded for each treatment.

When possible, hickory shad were implanted at the collection site because we feel that this reduces handling stress. Fish were landed with a 0.5 m round rubber net, the lure hook was removed and fish were placed in holding tanks of anesthetic. Males and females received an intramuscular (IM) implant of LHRHa in the dorsal musculature. Implants were administered through a spring-loaded 11-gauge syringe. Fish were placed in circular flow 3785 L tanks at 5 ppt salinity and transported to Manning Hatchery (Figure 1). Dissolved oxygen was continuously monitored and regulated (>7 ppm). Adults were either quick dumped or netted into 3.05 m natural spawn tank systems. A sex ratio of approximately 3:2 male/female is preferable in natural spawn systems. Salinity was maintained at 2-3 ppt. A 25% water change was performed each day to maintain adequate water quality. American shad were handled in the same manner as hickory shad with the exception that they were implanted at the hatchery. Fish spawned naturally and eggs were automatically transported to an egg collection box through an airlift system. Eggs were volumetrically measured (ml) and percent fertilization was determined. Eggs were placed in modified McDonald hatching jars supplied by approximately 2L/minute water flow. Prophylactic treatments of formalin were administered in the morning and afternoon to control fungus. Eggs were exposed to 600:1 treatment for approximately 20 minutes. Eggs began hatching at day six for American shad and day four for hickory shad. In order to stimulate a simultaneous hatch, jars were removed from the egg bank and placed outdoors in sunlight for ten minutes and stirred occasionally. The warm temperature, lower oxygen content, hormonal stimulation and agitation stimulated simultaneous hatching. Jars were then placed on 1.525 m circular flow-through larval tanks and water was flowed at approximately 2L/minute. Larvae flowed into tanks after hatch. Food was introduced to American shad at day three. Hickory shad feed on rotifers that are difficult to culture in the hatchery so hickory shad were marked and stocked before first feeding. American shad larvae were fed *Artemia* and 100µ AP100 three times daily. Prior to stocking, larvae were enumerated using a volumetric, direct proportion procedure in which a columnar sample of water was collected with a 25 mm diameter PVC tube at random locations in the larval tank. Larvae were enumerated in this sample and the total number of larvae in the tank was estimated by extrapolation to the total tank volume.

All stocked fish were given a hatchery mark to later identify a recaptured fish as hatchery origin. Shad that were stocked into study tributaries as larvae or early juveniles received an oxytetracycline (OTC) mark through either egg immersion, larval immersion or a combination of methods (INAD #9197). Shad stocked as late juveniles received an OTC feed mark and an internal numerical coded wire tag (CWT) (Northwest Technologies, Washington, USA).

Eggs were marked through hyperosmotic shock. This forces the uptake of OTC through the chorion, marking the larvae before hatch. Three-day-old eggs were placed in a neutral solution (buffered with sodium bicarbonate) of 1% OTC and 2.5% salt for ten minutes under heavy aeration. Several drops of foam inhibitor kept the solution from sudsing. Eggs were removed from the solution after the marking period and immediately rinsed in fresh water before return to the hatching jars.

Larval marks were produced by immersion in a 200 ppm buffered OTC bath for six hours.

Dissolved oxygen content was monitored and regulated (>5 ppm) by a carbon air stone connected to a liquid oxygen system (Table 2).

Table 2. *Summary of 2001 hyperosmotic egg marks and larval immersion marks with OTC.*

OTC Mark	Species	River
Egg	Hickory shad	Patuxent
Egg, day 3	Hickory shad	Choptank, Patuxent, Marshyhope
Day 1, 4	Hickory shad	Choptank
Egg, day 3, 6	American shad	Choptank
Day 3, 12	American shad	Patuxent
Day 3, 6	American shad	Patuxent
Day 9, 12	American shad	Patuxent

All water used at Manning Hatchery for OTC marking was softened (Culligan ion exchange system) before use in marking. Marking can only take place in water with a hardness below 20 mg/L and water hardness at Manning Hatchery routinely exceeds 200 mg/L. Marks were verified by viewing with a fluorescent microscope in the hatchery lab.

Larval stocking was accomplished by placing OTC marked larvae into boxes originally designed for shipping tropical fish. These containers consisted of an outer shell cardboard box, an inner foam box and a double thickness plastic fish bag. Larval tanks were drawn down to crowd the fish. Larvae were scooped out of the tanks and placed in the shipping bags/boxes, which were supplemented with approximately one ppt salt to ease stress. Each bag was filled with oxygen and sealed with electrician's tape. Boxes were driven to the stocking river and the bags were placed in the water long enough to temperature acclimate. The bags were then opened and river water was slowly introduced to further acclimate larvae to river water conditions. Bags were then emptied into flowing water to minimize predation.

Fish intended for early juvenile stocking were given larval immersion marks and stocked in hatchery ponds at six days of age. Fish were removed from ponds with seines and V-traps after approximately thirty days. Early juveniles were transported in fish hauling tanks at 3 ppt. salinity and D.O. saturation and quick dumped at the stocking sites.

Fish intended for late juvenile stocking in study tributaries were first placed in hatchery ponds at approximately three days of age. Mirant Mid-Atlantic (formerly Potomac Electric Power Company (PEPCO)) provides grow out facilities and manpower for the Department (Figure 1). Larvae were cultured in Mirant hatchery ponds for approximately 30 days. Fish were then trapped and transported to indoor grow out tanks for intensive culture. During this time all juveniles received an OTC feed mark for four consecutive days. Juveniles were reared to approximately 75 mm FL before implantation of CWT. Shad were transported from Mirant to Manning Hatchery in a 750 L tank for tagging. A one millimeter long CWT was implanted into each fish by dorsal insertion into the first pigment spot behind the operculum. Tagged fish were dropped through a tube and water flow carried the tagged fish through a quality control device that verified the tag retention and directed the animal to a stocking trailer tank. In addition to the tagging machine quality control device a mortality/tag retention study was performed each day fish were tagged in order to determine the actual number of tags available for recapture in field sampling. A random sample of 100 tagged fish were removed from the stocking trailer and placed in 2.44 m circular tanks at the hatchery in 3-5 ppt salinity. After seven days all live fish were checked for CWT retention, counted and recorded.

Juvenile stocking was accomplished by quick dumping tagged juveniles through a 15 cm hose directly from the transport trailer into the river. Juveniles are transported in approximately 3-5 ppt salinity to ease stress. D.O. was continuously monitored and regulated to saturation.

Results and Discussion

American shad strip spawning operations were disappointing due to a lack of brood fish present in spawning ground gill nets. Brood collection probably started too late in the season and the project missed the peak of the run. Production steadily declined from the first collection to the last collection (Table 3). The project collected 312 females that produced 2,250,000 eggs. Overall viability was calculated to 26%, which resulted in 582,000 larvae produced. In 2002 the project will fish its own nets with DNR biologists and two seasonal contractual employees. Fishing will commence the third week in March. Collection efforts will initially take place one to three times per week. As the run progresses toward its peak, collection will take place five days per week if tides are favorable for catching shad. More nets will be fished each night and different mesh sizes will be assessed. Other brood collection sites on the Potomac River and Patuxent River will also be investigated.

Table 3. *American shad brood fish and production data for 2001 strip spawn culture. Collections were conducted on the Potomac River at Marshall Hall.*

Date	Time Military	Tide	Ripe Females	Males	Total eggs collected	Percent Viability
04/20/01	1900	High	50	41	794,858	85.00%
04/25/01	1645	Low	29	11	114,120	50.00%
04/26/01	1715	Low	29	11	268,950	63.00%
04/27/01	1815	Low	30	11	234,927	66.00%
04/30/01	2100	Low	14	4	122,661	54.00%
05/01/01	1515	High	19	4	11,696	5.00%
05/02/01	1630	High	17	3	3,540	5.00%
05/03/01	1730	High	7	1	15,129	17.00%
05/04/01	1830	High	37	15	145,800	24.00%
05/07/01	2100	High	14	8	191,494	33.00%

Date	Time Military	Tide	Ripe Females	Males	Total eggs collected	Percent Viability
05/08/01	2145	High	5	2	19,040	20.00%
05/10/01	1730	Low	8	6	12,430	11.00%
05/11/01	1800	Low	17	10	1,776	1.00%
05/14/01	2015	Low	2	5	16,497	47.00%
05/15/01	2100	Low	12	4	158,351	67.00%
05/18/01	1745	High	13	6	18,534	9.00%
05/21/01	2000	High	9	1	120,414	40.00%
Totals			**312**	**128**	**2,250,217**	

Tank spawn production statistics are contained in Table 4. Larval American shad production and fertilization were lower in 1998-2001 compared to earlier years production (Table 5). American shad adults are not responding well to current tank spawning techniques compared to 1994-1997 production years. A cooperative effort with the University of Maryland Center of Marine Biotechnology is investigating the tank spawning effort. Dr. Yonathon Zohar is leading this work. Dr. Zohar is internationally recognized as an expert in this field and has assigned a graduate student to work with the Department. Experimental design for 2002 is currently being developed.

Table 4. *Tank spawn egg production statistics, 2001.*

	American shad	Hickory shad
Total eggs produced	4,578,491	31,248,495
Overall fertilization	15%	38%
Fertilized eggs produced	562,571	11,865,007
Total larvae produced	241,707	5,274,679

Hickory shad larval production and fertilization was lower than in previous years (Table 5). Hickory shad juvenile production was improved from previous years. This increase is attributable to refinements made in intensive culture procedures by Mirant staff.

Larval survival of both species in the hatchery tanks was poor. There is some evidence of water quality problems at Manning Hatchery, including super saturation of dissolved gases. This problem is under study and we hope to make adjustments before the 2002 season.

Table 5. *Annual stocking production in all tributaries, 1994-2001. The juvenile category includes fish stocked as early juveniles (late June) and as CWT juveniles (July/August). Fish were stocked in Choptank River, Patuxent River and Marshyhope Creek.*

Species	Year	Larvae	Juvenile
American shad	1994	1,240,000	14,240
American shad	1995	1,311,300	121,124
American shad	1996	2,367,600	289,104
American shad	1997	2,784,100	96,435
American shad	1998	227,200	33,611
American shad	1999	968,000	125,333
American shad	2000	731,000	128,414
American shad	2001	364,200	146,886
Hickory shad	1996	870,900	20,622
Hickory shad	1997	12,384,100	35,982
Hickory shad	1998	11,716,800	31,979
Hickory shad	1999	17,650,000	4,601
Hickory shad	2000	15,744,000	66,944
Hickory shad	2001	3,769,600	93,645

Hickory shad CWT tag loss and mortality was high in the first two trials. This was caused by a change in fish handling procedures resulting in stress mortality. During the July 26 trial, fish handling procedures were modified and performed as in past years. American shad tag loss and mortality due to implantation of CWT was very low in experimental trials (Table 6).

Table 6. *Results of a 2001 seven-day CWT retention and mortality study. Fish were held in hatchery tanks.*

Date	Species	% Survived + Tagged fish
July 25	Hickory shad	58%
July 25	Hickory shad	22%
July 26	Hickory shad	91%
July 30	American shad	91%
July 31	American shad	97%
August 1	American shad	93%

Hickory shad were stocked as larvae in the Patuxent River (Figure 2), Marshyhope Creek and Choptank River (Figure 3). Hickory shad were stocked as juveniles in Choptank River and Patuxent River. American shad larvae were stocked in the Patuxent River and the Nanticoke River. American shad were stocked as juveniles in the Choptank River and Patuxent River. Hickory shad larval stocking numbers were adequate and juvenile production was greater than expected. American shad larval and juvenile numbers were lower than anticipated (Tables 7 and 8). We have successfully produced more larvae and juveniles in previous years (Table 9). Early juveniles exhibited excellent survival during the period from pond stocking to river stocking. Survival for these fish over the period was 34% overall.

Figure 2. *2001 Patuxent River stocking sites.*

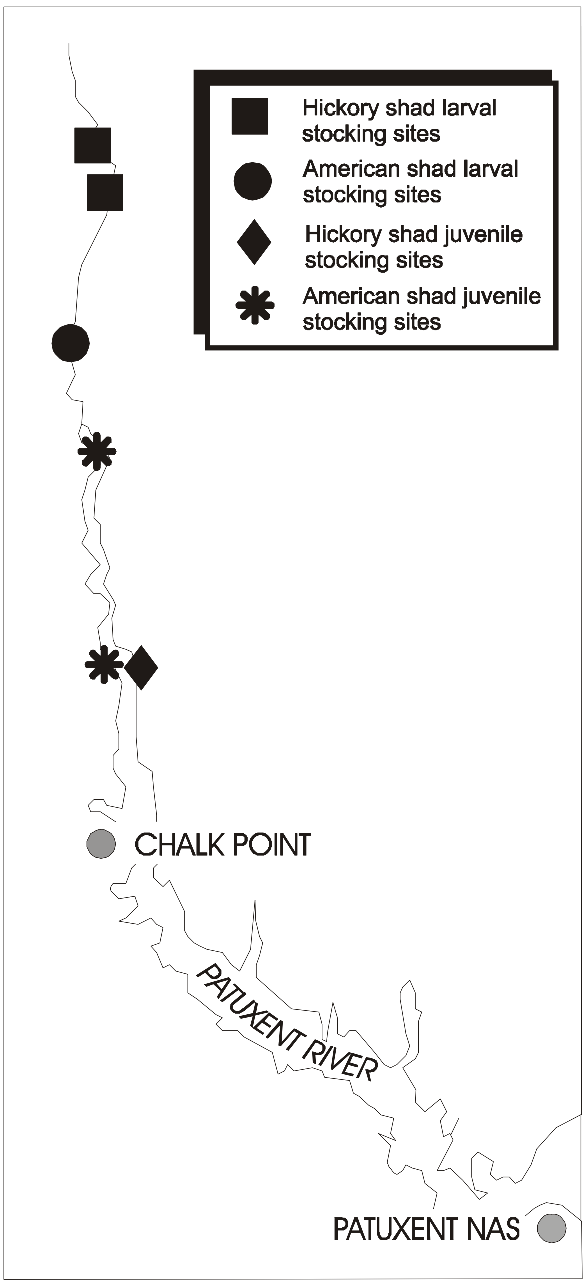
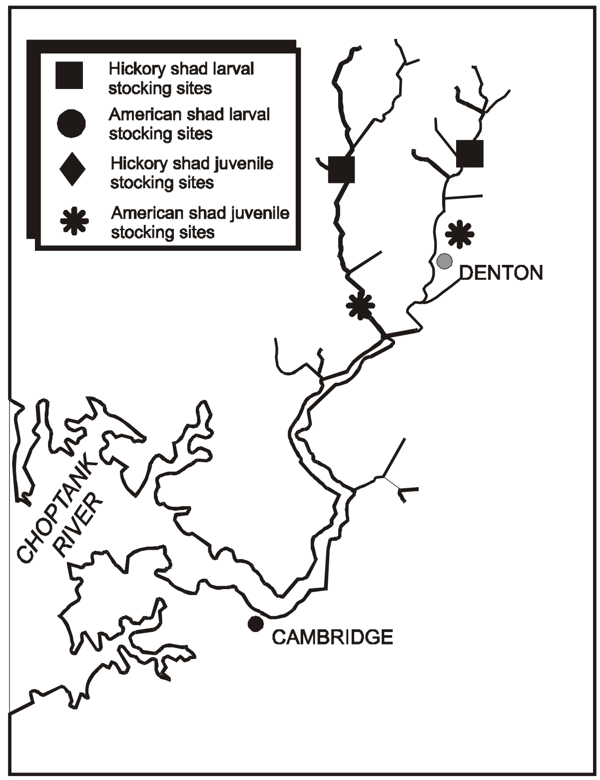


Figure 3. *2001 Choptank River stocking sites.*



Due to the inconsistent availability of EVAC GnRHa implants available on the commercial market, the program took the decision to manufacture hickory shad implants in our lab. The implants we manufactured performed identically to commercially prepared cholesterol product at reduced cost. Commercial cholesterol implants cost $6.00-12.00/implant. We were able to manufacture cholesterol implants at a unit cost of approximately $1.00. Unfortunately the cholesterol based implant is not compatible with efficient American shad tank spawning compared to EVAC-based implants, which have proven highly effective in past years. The commercial supplier of EVAC implants for American shad will not manufacture product for 2002. The staff at UMD COMB will manufacture implants for 2002. Sources for EVAC LHRHa implants after 2002 are unknown.

Samples analyzed from each group of OTC egg and larval marked fish indicated that all fish stocked were successfully marked.

Figure 4. ***N****anticoke River drainage stocking sites in 2001.*

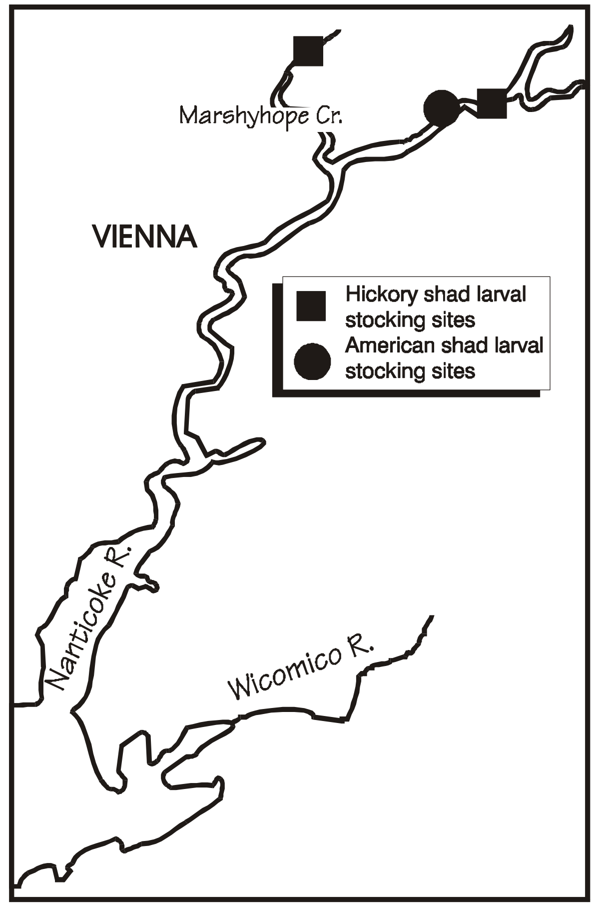


Table 7. *Stocking events in 2001.*

Species	Life stage	Date	River	Number stocked
American shad	Larvae	May 9	Mirant Ponds	226,300
American shad	Larvae	May 10	Patuxent	142,700
American shad	Larvae	May 10	Cedarville Ponds	95,000
American shad	Larvae	May 11	Cedarville Ponds	45,000
American shad	Larvae	May 16	Mirant Ponds	50,000
American shad	Larvae	May 23	Cedarville Ponds	17,000
American shad	Larvae	May 23	Cedarville Ponds	3,800
American shad	Larvae	May 25	Cedarville Ponds	10,000
American shad	Larvae	May 30	Mirant Ponds	100,000
American shad	Larvae	June 4	Patuxent	221,500
American shad	Larvae	June 4	Nanticoke	40,000
American shad	Early juvenile	June 5-12	Patuxent	77,500
American shad	Early juvenile	June 7	Choptank	10,500
American shad	Early juvenile	June 4	Choptank	4,500
American shad	CWT Juvenile	July 30	Patuxent	14,740
American shad	CWT Juvenile	July 31	Choptank	12,377
American shad	CWT Juvenile	July 31	Patuxent	7,163
American shad	CWT Juvenile	August 1	Choptank	20,106
Hickory shad	Larvae	April 20	Patuxent	89,600
Hickory shad	Larvae	April 24	Choptank	870,000
Hickory shad	Larvae	April 24	Patuxent	910,000
Hickory shad	Larvae	April 26	Mirant Ponds	250,000
Hickory shad	Larvae	April 27	Marshyhope	1,230,000
Hickory shad	Larvae	April 27	Nanticoke	1,230,000
Hickory shad	Larvae	April 30	Choptank	241,400
Hickory shad	Larvae	April 30	Choptank	47,400
Hickory shad	Larvae	May 2	Patuxent	381,200
Hickory shad	Early juvenile	May 29-June 16	Patuxent	53,500
Hickory shad	CWT juvenile	July 17	Patuxent	13,561
Hickory shad	CWT juvenile	July 18	Patuxent	6,677
Hickory shad	CWT juvenile	July 18	Choptank	8,446
Hickory shad	CWT juvenile	July 19	Choptank	11,461

Table 8. *Breakdown of 2001 juveniles stocked by phase.*

Species	Stocking Phase	River	Number stocked
American shad	Early Juvenile	Patuxent River	77,500
American shad	Early Juvenile	Choptank River	15,000
American shad	CWT Juvenile	Patuxent River	21,903
American shad	CWT Juvenile	Choptank River	32,483
Hickory shad	Early Juvenile	Patuxent River	53,500
Hickory shad	CWT Juvenile	Patuxent River	20,238
Hickory shad	CWT Juvenile	Choptank River	19,907

Table 9. *Historical stocking statistics for larval and juvenile American shad and hickory shad in target tributaries since the inception of the restoration effort (1994-2001).*

Species	River/ Life stage	1995	1996	1997	1998	1999	2000	2001
American shad	Patuxent Larvae	346,000	655,000	1,345,000	61,000	526,000	349,000	364,188
American shad	Patuxent Early juv						37,250	77,500
American shad	Patuxent Juvenile	121,124	173,994	60,040	16,726	60,377	26,765	21,903
American shad	Choptank Larvae		626,000	1,245,000	136,000	442,000	357,000	
American shad	Choptank early juv							15,000
American shad	Choptank Juvenile		115,110	32,612	16,885	64,596	64,399	32,483
American shad	Nanticoke Larvae			152,000				40,000
Hickory shad	Patuxent Larvae		746,000	5,118,000	6,475,370	8,106,000	8,235,000	1,380,800
Hickory shad	Patuxent early juv.							53,500
Hickory shad	Patuxent Juvenile		12,659	35,982	31,979	4,601	28,436	20,238
Hickory shad	Choptank Larvae		125,000	5,571,000	4,991,000	8,719,000	5,634,000	1,158,800
Hickory shad	Choptank Juvenile		7,963				38,508	19,907
Hickory shad	Marshyho pe larvae							1,230,000
Hickory shad	Patapsco larvae				1,695,000	250,000	825,703	500,000

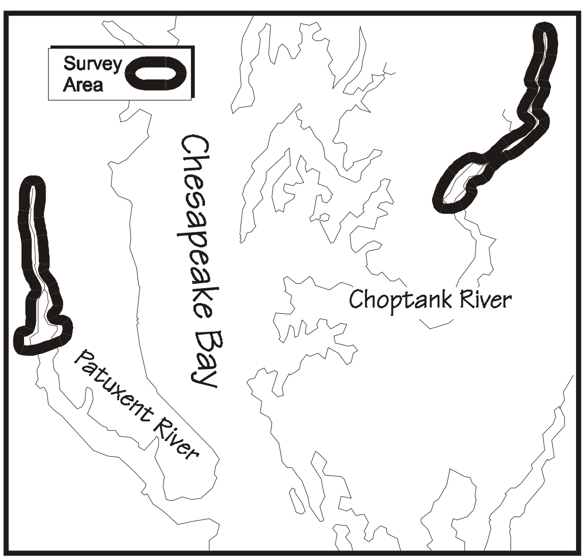
Sub-project 2

A. "Monitor the abundance and mortality of larval and juvenile shad using marked hatchery-produced fish"; B. "assess the contribution of hatchery-produced fish on the resident/pre-migratory stock in Chesapeake Bay tributaries"

Materials and Methods

Post-larval and pre-juvenile hickory shad were sampled by trawl. Trawls were performed weekly (Figure 5) from June 5 through June 25, 2001 in the Patuxent River and from June 12, 2001 through June 26, 2001 in the Choptank River. Trawls spread mid-water at 1.5m horizontally and vertically in tow. Head rope, foot rope and wing ropes hung at 2.75m. Net was nylon and constructed of 32mm stretch mesh dog-ears of #9 thread and 13mm stretch mesh body of #147 knotless nylon. Head and foot ropes were 10mm diameter Poly-dac net rope with legs extended 3m and wire rope thimbles spliced in at each end. Five 76mm x 127mm plastic floats were attached to head rope. Footrope was hung loop style with 3mm galvanized chain. One 16mm depressor was rigged to each wingtip on footrope. Doors were Super Krub, 30cm x 41cm and fully rigged with 30m of 3mm diameter nylon rope. Trawl samples were kept on ice in the boat and frozen within 3 hours of collection. Alosid species were picked from these samples in the lab and identified. Sagittal otoliths were removed and mounted on slides with Crystalbond 509 (Aremco Products, Ossining, NY). Otoliths were lightly ground on 600 grit silicon carbide wet sandpaper and viewed under 400x magnification under epifluorescent light at 50 watts with a Zeiss Axioskop 20 microscope. The presence and location of fluorescent marks was recorded.

Figure 5. *Area surveyed by mid water trawl in 2001.*



Juvenile shad were collected by seine weekly from August 8 through October 2, 2001 in the Patuxent River (Figure 6) and from August 9 through October 3, 2001 in the Choptank River (Figure 7). A seine 61 meters in length, 3.1 meters in height, and 6.4mm mesh, was set by boat and pulled to shore at established seine sites. Juvenile American and hickory shad were picked from the collection and placed on ice in the boat, then frozen upon return to the lab. These samples were thawed, measured (fork length in mm) and scanned for presence of numeric wire tags (CWT). If CWT were present, they were removed by dissection and examined under the microscope for identification. Sagittal otoliths were dissected from every individual and mounted on slides with Crystalbond 509, and examined in the same way as post-larval/pre-juvenile shad.

Figure 6. Juvenile seine survey sites in the Patuxent River, 2001.

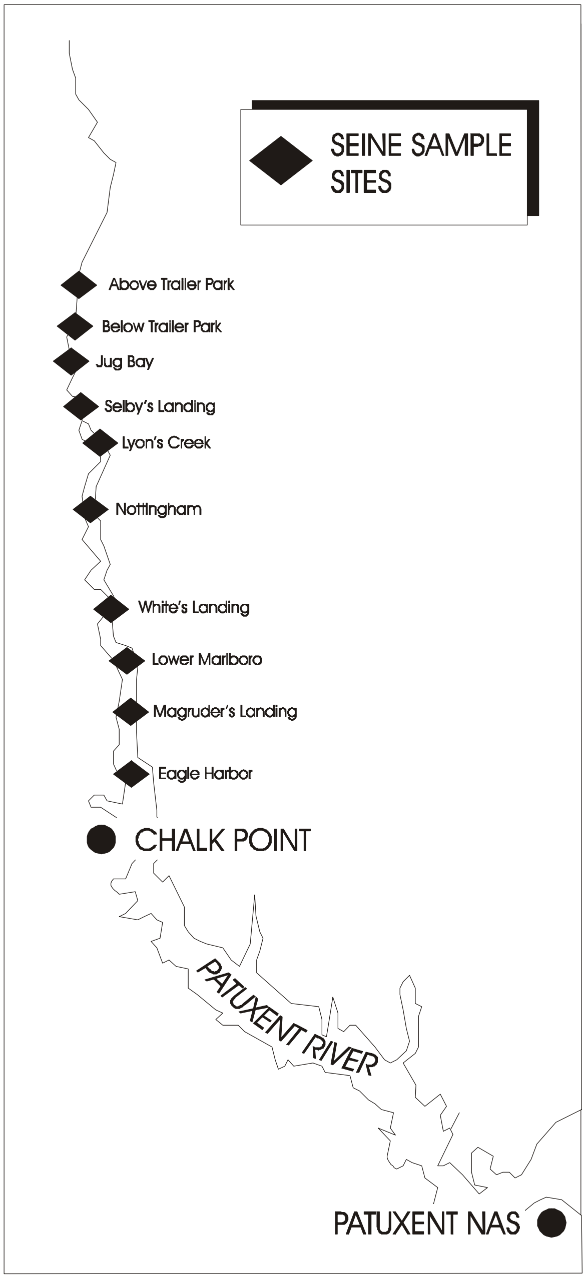
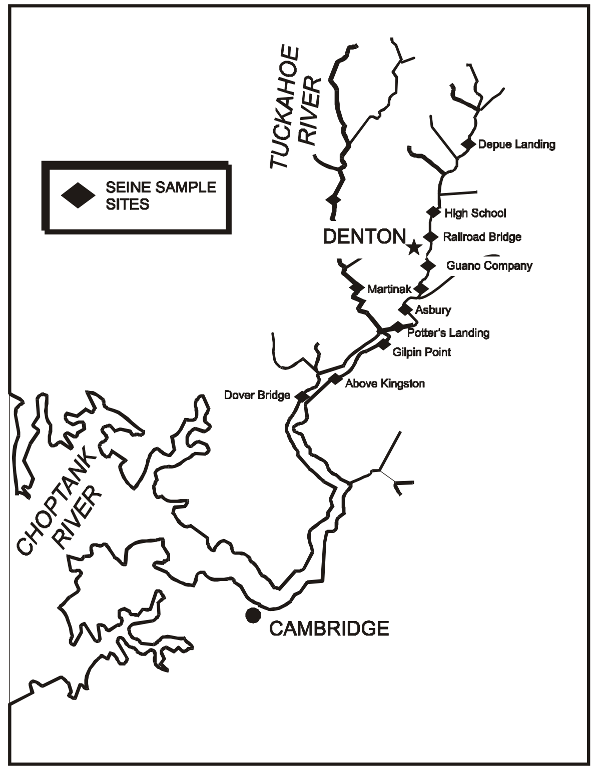


Figure 7. Juvenile seine survey sites in the Choptank River, 2001.



Estimates of juvenile abundance, mortality and survival were derived from the following:

Larval survival to CWT juvenile stocking was calculated by (Ricker 1975):

$$S_1 = (R_{12})\, M_2 / (M_1)\, R_{22}$$

$$\text{variance } S_1 = S_1^2 \{(1/R_{12}) + (1/R_{22}) - (1/M_1) - (1/M_2)\}$$

where M_1 is the number of fish marked at the start of the first interval, M_2 is the number of fish marked at the start of the second interval, R_{12} is recaptures of first interval marked fish in the second interval, R_{22} is recaptures of second interval marked fish in the second interval and S_1 is the survival rate during interval one (from the time of marking in interval one to time of marking in interval two). All data was adjusted for CWT loss and mortality using data obtained in tag retention trials.

Instantaneous mortality is derived from survival estimates and is used in conjunction with stocking data to calculate juvenile abundance:

$$Z = -\ln S_1 / interval$$

where Z is instantaneous mortality rate and S_1 is survival rate.

Abundance of juvenile shad prior to out migration was also calculated by Chapman's modification to the Peterson estimate (Ricker 1975):

$$N = \{(C+1)\ (M+1)\} / (R + 1)$$

where N is the population estimate, M is the number of marked fish stocked, C is the number of fish examined for tags (total recaptures) and R is the number of marked fish that were recaptured.

From Ricker (1975): Calculation of 95% confidences limits based on sampling error using the number of recaptures in conjunction with Poisson distribution approximation.

Chapman's modification (1951):

$$N^* = \{(C+1)\ (M+1)\} / (R_1 + 1)$$

where R_1 is from Pearson's formula to calculate upper and lower limits:

$$R_1 = R + 1.92 \pm 1.960\sqrt{R + 1.0}$$

Results and Discussion

The Patuxent River mid-water trawl survey captured only seven juvenile hickory shad (Table 10). Five of these fish were wild origin and two fish were identified as hatchery origin by otolith mark assessment. The Choptank River mid-water trawl survey caught no juvenile hickory shad. The trawl survey was originally proposed in order to capture juvenile hickory shad that were rarely captured by the seine survey. As populations of hickory shad are increasing, hickory shad juveniles are appearing more frequently in seine collections. Due to the lack of data and information generated by the trawl survey, we will not sample with a mid-water trawl in 2002. The seine survey should provide more valuable data and the trawl survey is not cost effective or a wise use of manpower.

Table 10. *Hickory shad juveniles collected in 2001 by mid-water trawl in the Patuxent River.*

Patuxent River	6/05	6/13	6/19	Site totals:
Trailer Park	0	0	0	0
Western Branch	1	0	0	1
Selby Lndg.	0	1	1	2
Lyons Ck.	0	0	0	0
Deep Turn	0	0	2	2
Bowling Lndg.	0	0	0	0
A. Lw. Marlboro	0	0	0	0
Whites Lndg.	1	0	1	2
Kings Lndg.	0	0	0	0
Eagle Harbor	0	0	0	0
Daily totals:	2	1	4	7

This study collected 327 American shad juveniles by seine in the Patuxent River (Table 11) in 2001. An additional 75 samples were collected by a striped bass seine survey conducted by another program on 3 dates (7/19, 8/16 and 9/13/01) and these samples were also examined. Of these 402 American shad, 401 pairs of sagittal otoliths were successfully extracted, mounted on slides, polished, and examined under UV microscopy for epifluoresence. Otolith examination determined that of the 326 otolith pairs examined from our juvenile collections, 36 (11.04%) were unmarked juveniles from natural reproduction. The remaining 290 juveniles (88.96%) originated from our hatchery production. Of this 290 shad of hatchery origin, 49 (16.90%) were identified from OTC marks on otoliths as larval stocked. American shad stocked as "early juveniles" from June 5 - 12, 2001 totaled 161 (55.52%) and CWT - tagged juveniles stocked July 30 - 31, totaled 80 (27.59%). Of these 80 specimens, 7 had lost their tags but were identified by the hatchery feed marks imparted to the otoliths. The tag retention of CWT juveniles from tagging until the end of this study is 91.25%. This data supports our estimate of CWT loss calculated from tank holding experiments conducted at stocking. When the samples provided to us by the striped bass survey were examined, we found similar results. When 25 American shad samples were collected on July 19, 2001 (before our CWT juveniles were stocked), 8 were found to be "wild" (32%), one was from our larval stocking (4%), and the remaining 16 (64%) were found to have been stocked as "early juveniles". Of the remaining 50 American shad collected by the striped bass survey after we stocked CWT - juveniles, 16 (32%) were found to be "wild". Of the remaining 34 fish of hatchery origin, 33 were stocked as "early juveniles", and one as a CWT juvenile that did not retain its tag. For the third consecutive year, American shad stocked as juveniles are highly represented in the seine surveys. Wild American shad juveniles were collected in the Patuxent River for the fourth consecutive year, indicating increasing natural reproduction.

Table 11. *Number of American shad juveniles collected in the 2001 Patuxent River seine survey.*

	8/08	8/14	8/22	8/28	9/04	9/18	9/25	10/02	Site total
Patuxent R. sites:									
Above Trailer Park	3	2	2	1	0	0	2	0	10
Below Trailer Park	9	0	3	3	0	1	1	0	17
Jug Bay	2	1	0	1	0	0	1	2	7
Selby Landing	7	15	12	19	3	3	9	7	75
Lyon's Creek	3	1	14	2	20	6	0	3	49
Nottingham	21	1	8	1	27	17	1	1	77
White's Landing	2	1	2	1	11	1	6	2	26
Lower Marlboro	0	0	4	5	2	1	0	2	14
Magruder's Landing	10	2	9	7	16	8	0	0	52
Eagle Harbor	0	0	0	0	0	0	0	0	0
Daily totals	**57**	**23**	**54**	**40**	**79**	**37**	**20**	**17**	**327**

We collected 41 American shad juveniles by seine in the Choptank River in 2001, compared with only seven collected at the same seine sites in 2000. Seven (17%) of the 41 samples displayed no marks and are "wild" or resulting from natural reproduction. The remaining 34, or 82.93%, are of hatchery origin; 21 of which were stocked as CWT- juveniles and 13 as early juveniles. Tag retention for CWT stocked juveniles was 90.48% in the Choptank River.

Thirty-one juvenile hickory shad were collected by seine in the Patuxent River in 2001, a slight decrease from 37 collected in 2000 (Table 12). An additional three hickory shad juveniles collected on 9-13-01 at Milltown Landing were provided to us by the juvenile seine striped bass survey. Twenty-one (62%) of these 34 juvenile hickory shad were found to be unmarked "wild" fish from natural reproduction. The remaining 13 (38%) were marked or tagged hatchery-produced fish, nine of which were "early juveniles" and four were CWT-marked. As in previous years, the White's Landing seine site produced more hickory shad samples than any other. Although sample sizes were small for both years, far more wild hickory shad juveniles were collected in 2001 (21 or 61.76% of total) than in 2000 (14 or 37.84%). Hickory shad juveniles are more difficult to capture than American shad juveniles. We are cautiously (considering small sample sizes) optimistic that these data suggest hickory shad restoration is progressing well in the Patuxent River.

Table 12. *Location of hickory shad juveniles collected in the 2001 Patuxent River seine survey.*

Patuxent R. sites	8/08	8/14	8/22	8/28	9/04	9/18	9/25	10/2	Total
Above Trailer Park	0	0	0	0	0	0	0	0	0
Below Trailer Park	0	0	4	0	0	0	0	0	4
Jug Bay	0	0	0	0	0	0	0	0	0
Selby Landing	0	0	0	0	0	0	0	1	1
Lyon's Creek	0	0	0	0	4	1	0	0	5
Nottingham	0	0	0	0	2	0	1	1	4
White's Landing	0	2	0	1	0	4	3	0	10
Lower Marlboro	0	0	0	0	0	1	0	0	1
Magruder's Landing	0	1	0	2	0	3	0	0	6
Eagle Harbor	0	0	0	0	0	0	0	0	0
Date totals:	**0**	**3**	**4**	**3**	**6**	**9**	**4**	**2**	**31**

In addition to providing future brood fish, juvenile stocking is valuable as a pre-migratory stock assessment tool through utilization of multiple mark-recapture techniques. It will also help us evaluate the efficacy of stocking different life stages of fish on the eventual returns to the adult population. Calculation of survival of stocked fish in conjunction with adult return data will enable cost-benefit analysis of larval vs. juvenile stocking.

Estimates of survival, instantaneous mortality and abundance were calculated for American shad on the Patuxent River. Since no larvae were stocked in the Choptank River, no estimate of larval mortality could be calculated. Estimates of abundance of Choptank River juvenile American shad were calculated.

Survival of larval stocked American shad to CWT juvenile stocking in the Patuxent River was calculated to 0.0338 for the 81day period. Survival of early juvenile stocked shad to CWT juvenile stocking was calculated to 0.6292 for the 55-day period.

Daily mortality of larval stocked shad to the time of CWT juvenile stocking was calculated to 4.2% (Z=0.0418/d, ±2 SE=0.0122) for the 81-day interval. Daily mortality of early juvenile stocked shad to the time of CWT juvenile stocking was calculated to 0.8% (Z=0.0084/d, ±2 SE=0.1662) for the 55-day interval.

Juvenile abundance of larval stocked American shad (July 30 2001) was calculated at 12,317 using survival estimates and stocking data. Early juvenile abundance was calculated using survival estimates and stocking data and estimated at 48,765 on July 30, 2001.

Total juvenile abundance in the Patuxent River (July 30, 2001) was calculated by Chapman's modification to the Peterson estimate at 94,771 (upper limit=117,789, lower limit=76,255). Using survey recapture rates we can estimate the composition of the juvenile stock on July 30 (Table 13). Particularly noteworthy was the excellent survival of early juvenile stocked fish. Total survival of these fish from pond stocking (larvae) to July 30 (juvenile) was 22%. Only 3% of larval fish stocked directly into the river over the same period survived to July 30. 150,000 larvae stocked in Mirant ponds and grown to juvenile size for CWT tagging were estimated to have a survival of 14.6% for the period from pond stocking to July 30 (21,903 juveniles). The results from this preliminary work on early juvenile stocking are encouraging and will be further investigated in 2002. Previous years data has indicated that American shad year class success is largely set by CWT juvenile stocking size and mortality of juvenile shad is very low (1-2%) from 75 mm size to fall out migration. It is not known how well these fish stocked as early juveniles will imprint on the river of stocking origin. We will continue to stock early juveniles until their stream fidelity can be assessed through sampling for returning adults (Project 3).

Table 13. *Estimates of American shad juvenile abundance in the Patuxent River on July 30, 2001. Figures were calculated using Chapman's modification to the Peterson equation (95% confidence interval).*

Life stage	Peterson Estimate	Upper Limit	Lower Limit
Larval stocked	12,351	15,350	9,937
Early juvenile stocked	48,898	60,774	39,344
CWT juvenile stocked	20,416	25,375	16,427
Wild juveniles	13,107	16,290	10,456
Totals	94,771	117,789	76,255

Juvenile abundance has been calculated annually for the Patuxent River since 1995 (Table 14). Total juvenile abundance is variable according to the level of stocking effort/survival for each year and is positively correlated with larval-origin juvenile abundance ($r^2 = 0.73$, $P < 0.015$). Larval-origin juvenile abundance is not correlated with larval stocking effort however. This is due to variable larval survival from year to year. Total abundance appears to be impacted by a combination of larval survival and stocking effort of both juveniles and larvae. As natural reproduction increases, the effects of stocking and larval survival on the total juvenile abundance should diminish. While total abundance does not necessarily increase each year, wild abundance has exhibited a positive trend. 2001 data indicates a large increase in the number of wild juveniles compared to all previous years.

Table 14. *1995-2001 American shad summer juvenile abundance estimates in the Patuxent River. Figures were calculated using Chapman's modification to the Peterson equation (95% confidence interval, numbers may not add up due to rounding).*

Year	Larval Stocked Origin	Juvenile Stocked Origin	Wild Origin	Total Juveniles
1995	78,500	73,300	0	151,800
1996	81,000	153,600	0	234,600
1997	116,000	48,100	0	164,100
1998	0	9,200	1,800	11,000
1999	18,000	53,200	800	72,000
2000	700	43,200	3,600	47,600
2001	12,300	69,300	13,100	94,800

Natural recruitment is beginning to occur in the Patuxent River according to the abundance estimates. No wild juveniles were captured in the first four years of the restoration effort (Table 15). 1998 sampling captured 17% wild fish but sample numbers were very low. Abundance of wild juveniles in the nursery area in 1998 was estimated at less than 100 fish (Minkkinen et al 1999). Poor hatchery production in 1998 resulted in low stocking numbers for juvenile and larval American shad. In 1999 and 2000 there were high numbers of recaptures and wild component increased over the period. Hatchery-origin females from initial stockings are nearing full recruitment to the returning spawning stock. In 2001 wild juveniles increased dramatically in the Patuxent River. We anticipate that adult returns will continue to grow as more stocked year classes become fully recruited. The wild component of the juvenile population should increase accordingly. We will continue to monitor the success of our restoration efforts through assessment of origin composition of the juvenile population.

Table 15*. Juvenile American shad recaptures in Patuxent River from summer seine survey since inception of the restoration effort, 1994-2001 Data is percentage of origin composition of all juveniles collected by the survey. N=number of recaptured juvenile American shad.*

Sample year	N	Larval stocked origin	Juvenile stocked origin	Wild fish
1994‡	NA	0%	100%	0%
1995	330	54%	46%	0%
1996	285	60%	40%	0%
1997	362	79%	21%	0%
1998	90	0%	83%	17%
1999	260	25%	74%	1%
2000	340	1%	91%	8%
2001	376	13%	73%	14%

‡Data collected from a related trawl survey. Seine survey began in 1995.

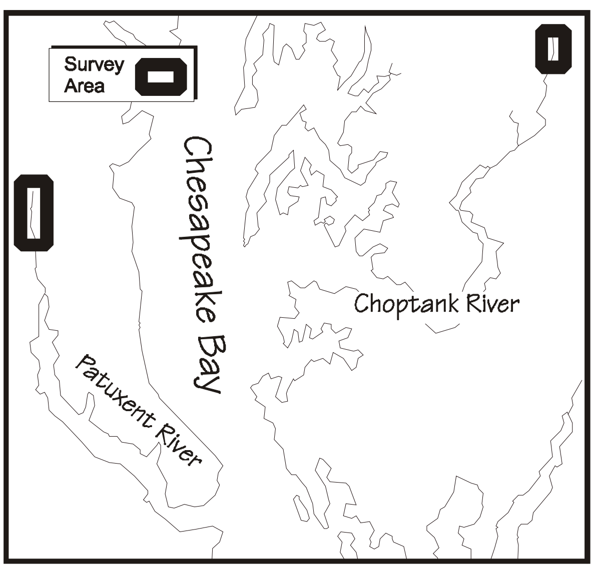
Sub-project 3.
Estimate the contribution of hatchery fish to the adult spawning population and monitor recovery of naturally produced stocks.

We conducted a Patuxent River and Choptank River spawning ground survey in 2001 to collect adult American shad and hickory shad. The contribution of hatchery fish to the spawning populations was investigated. We hope that high numbers of hatchery fish on the spawning grounds will stimulate natural recruitment to the population, something that has not occurred in decades. Restorative stocking of American shad began in 1994, so we expected adult returns to these rivers in 2001 at age seven and younger. Hickory shad stocking began in 1996 and we expected increasing hickory shad adult returns in 2001.

Methods and materials

The survey was conducted with a Smith Root electro-fishing boat. Some supplemental angling and creel survey was conducted upstream of where we could travel by boat. Sampling was conducted in historical spawning areas described by anecdotal data and concentrated in river sections where shad were encountered in 1999 and 2000 sampling (Figure 8). Adults were encountered in both rivers in areas that displayed similar physical characteristics. Sites are generally characterized as occurring from the uppermost areas experiencing any tidal effects to the lowermost, non-tidal areas of the rivers. In the Patuxent River, this area was from two miles upstream of Wayson's Corner (sewage plant) to Queen Anne Bridge. In the Choptank River, shad were captured from above the Route 313 Bridge in Greensboro, Maryland to approximately two miles upstream. A popular fishing spot at Red Bridges (one mile above uppermost electro-fishing site) was creel surveyed several times and limited shad captures were reported. The Tuckahoe River is a major tributary of the Choptank River that we have stocked. Recreational anglers made adult collections on the Tuckahoe River since access by electro fishing boat is precluded. Several fishermen were supplied with collection permits and agreed to save a sub-sample of shad that were captured. Most Tuckahoe River samples were collected just below Crouse Mill Dam in Tuckahoe State Park. These samples are considered to be Choptank River origin for the purposes of data analysis since the Tuckahoe River stocked fish do not receive a unique mark from Choptank River fish. It is likely that shad also utilize tidal freshwater areas downstream of our collection sites, but increasing river depth reduced our capture efficiency with electro fishing gear.

Figure 8. *Area sampled by electrofishing boat in 2001 adult sampling survey.*



Surveys were conducted in April and May when water temperatures were between 10° and 21° Celsius (Figures 9 and 10). Hickory shad were captured between April 2 and May 31. Most were captured between April 10 and May 8. American shad were encountered from April 4 until May 31. Most were captured between April 26 and May 16. Hickory shad are reported to spawn in river temperatures between 52° and 65°F. Recaptures reflect this temperature regime. American shad were encountered when river temperatures were between 12° and 21°C, which reflects the preferred spawning temperature for this species.

Figure 9. *2001 Patuxent River adult hickory shad and American shad electrofishing captures. Solid line indicates river temperature.*

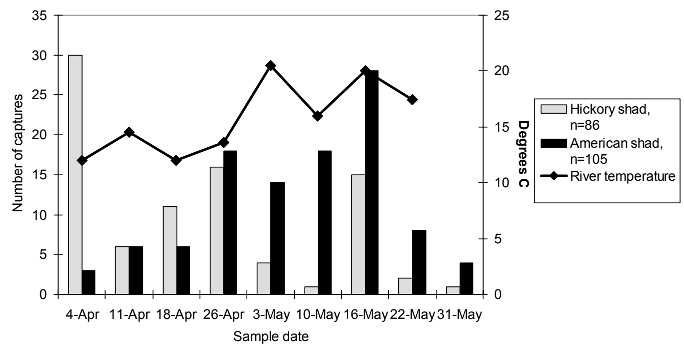
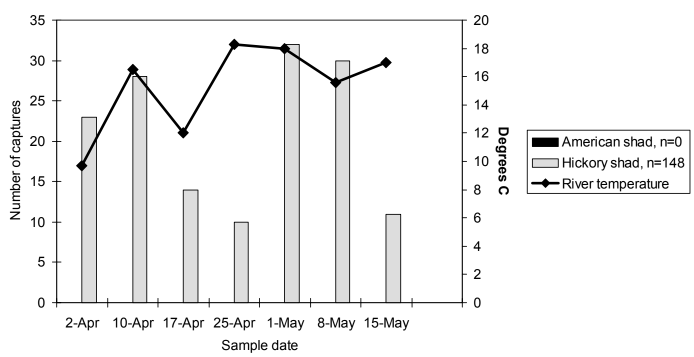


Figure 10.*2001 Choptank River adult hickory shad and American shad electrofishing captures. Solid line indicates river temperature.*



Fish collected were processed in the following manner. Length (FL) and sex were recorded. A scale sample was taken for age analysis and otoliths were extracted to look for hatchery OTC marks. All stocked American shad are marked with river-specific patterns. This allows us to collect information on hatchery contribution and river fidelity of American shad. Stocked hickory shad are marked to identify the fish as hatchery origin and all had a common mark among river systems. The fish were also scanned for CWT that are implanted in fish stocked as juveniles. CWT data allow for analysis of specific stocking events and age validation studies.

Results and discussion

Hickory shad collected in each river were from both wild and hatchery origin. Hatchery fish comprised 76% of the hickory shad analyzed from the Patuxent River and 68% in the Choptank River. Hatchery fish were collected throughout the time period of the run (Figures 11 and 12).

Hickory shad scales were cleaned, mounted between glass slides and aged using a microfiche reader. In the Choptank River all adults were estimated to be between 2 and 5 years of age (Figure 13). Both wild and hatchery females were from the 1997 and 1998 year class (Figure 14). Wild males were represented from the 1996 to 1999 year classes (Figure 15). Hatchery males were from 1996 through 1999 stockings. Looking at the sexes-combined data, age three is the predominate age of return, representing 76% of the fish collected. Age two fish comprised 1.4% of the population. Age four and greater represented 22.5% of the fish aged.

In the Patuxent River, wild females were from the 1995 to 1998 year classes and hatchery fish from the 1996 to 1998 stocking (Figures 16-18). For males, wild fish originated from 1996 through 1998 year classes and hatchery fish were from the 1996 to 1998 year class. With sexes-combined data, age two fish comprised 1.2%, age three comprised 65.1% and ages four and greater fish comprised 33.7% of the sample.

A total of 105 American shad collected in the Patuxent River were also comprised of wild and hatchery fish (Figures 19 and 20). OTC marks were present on 93% of the fish examined (Figure 21). In 1999 60% were of hatchery origin and in 2000 hatchery fish represented 91% of the sample. This data indicates that hatchery fish are contributing greatly to the preexisting remnant population in the Patuxent River. Only two wild females were captured (1995, 1996 year classes). Twenty-seven hatchery females were represented from the 1995 to 1998 year classes indicating that they have begun recruiting into the spawning population. Females represented 35% of the captures in our survey. Wild males were represented from the 1994 to 1997 year classes. Hatchery males were represented from the 1995, 1996, 1997 and 1998 juvenile and larval stocking events. The contribution of juvenile, larval and wild origin fish by year class indicates the increasing contribution of hatchery fish each year from 1994 to 1997. Hatchery fish comprised 0% of the 1994 year class, 88% of the 1995 year class, 92% of the 1996 year class and 97% of the 1997 year class (Figure 22). Five American shad were collected in the Choptank River. OTC marks were present on 100% of the fish examined. No hatchery females were captured. Females represented 0% of the Choptank River captures. Hatchery males were represented from the 1996 to 1998 year classes. The contribution of Choptank River juvenile, larval and wild origin fish by year class indicates the absence of any viable natural spawning population. Hatchery fish comprised 100% of 1998 year class fish, 100% of 1997 year class fish and 100% of 1996 year class fish.

Success of this program relies on eventual natural recruitment from hatchery-produced adults.

There has been no measurable recruitment in the Choptank River or Patuxent River since the 1970's (Maryland Juvenile Recruitment Survey). We have conducted an intensive juvenile survey in the Patuxent River since 1994 and the Choptank River since 1996 (sub-project 2). No wild American shad were captured in these surveys until 1998. During 1999, 1% of the Patuxent River juveniles examined were wild. Eight percent of the Patuxent River juveniles captured in 2000 were wild and 14% were wild in 2001. As the wild component of the population increases we will use mark-recapture techniques to estimate the level of natural recruitment in these rivers. We will evaluate this data to determine when restoration goals have been satisfied.

We have used the Susquehanna River/Deer Creek hickory shad population as a source of brood fish for restoration efforts. This population declined along with other stocks during the 1970's, but had experienced a mild resurgence during the 1980's. During 1993 recruitment to this population suddenly increased. This year class provided us with a sufficient source of adults when they began returning in 1996. Hickory shad are short-lived compared to American shad. Aging studies indicate that these fish begin to return at age two, with age three fish predominating and few fish remaining at age four and older (Batsavage 1997). We have compiled data about the age structure of this population during each year of brood fish collection (Figure 23). Our data indicate that age three fish are predominant in the population. The contribution of age four and older fish increased during 1997 and 1998 as the 1993 year class matured. This is indicated by the expanding length frequency distribution of the stock (Figure 24). During 1999 recruitment from the strong 1996 year class caused the contribution of older fish to decline in relation to age three fish. This recruitment data and observational data of the numbers of adults on the Susquehanna River spawning grounds indicate an expanding population.

The restoration effort is beginning to show some positive results. Hatchery adults are returning to spawn in increasing numbers. Hatchery origin adults dominate the spawning grounds (Figure 25). Electro fishing CPUE (fish/hour of fishing time) for hickory shad was 0.309132 in the Patuxent River and 0.993087 in the Choptank River (Figure 26). Patuxent River CPUE for American shad was 0.310993. No American shad were collected in the Choptank River (Figure 27). Wild juvenile recruitment is also increasing. Our observations and CPUE data indicate rapidly increasing population levels. Increasing angler participation also supports this trend as anglers begin to target these fish after incidentally catching them. We expect to see the adult populations comprised mostly of hatchery fish for several years. A healthy spawning run of adult fish should result in an increasing wild component in the juvenile population. As these wild juveniles return as spawning adults we should see a corresponding increase in the wild component of the adult spawning population.

Figure 11. 2001 Patuxent River adult hickory shad collections. Bars indicate proportion of hatchery fish to wild fish over the sample season indicated by successful processing of otolith samples. Due to their fragile nature, hickory shad otoliths are frequently damaged in processing.

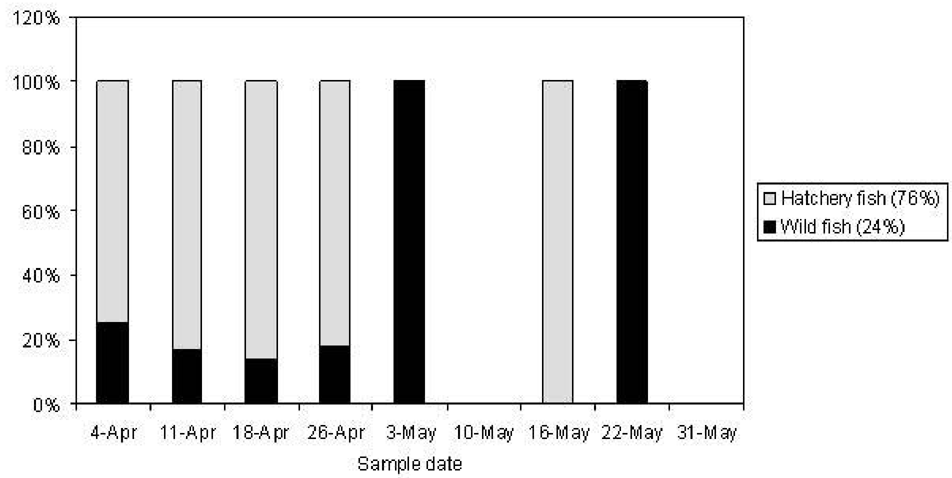


Figure 12. 2001 Choptank River adult hickory shad collections. Bars indicate proportion of hatchery fish to wild fish over the sample season.

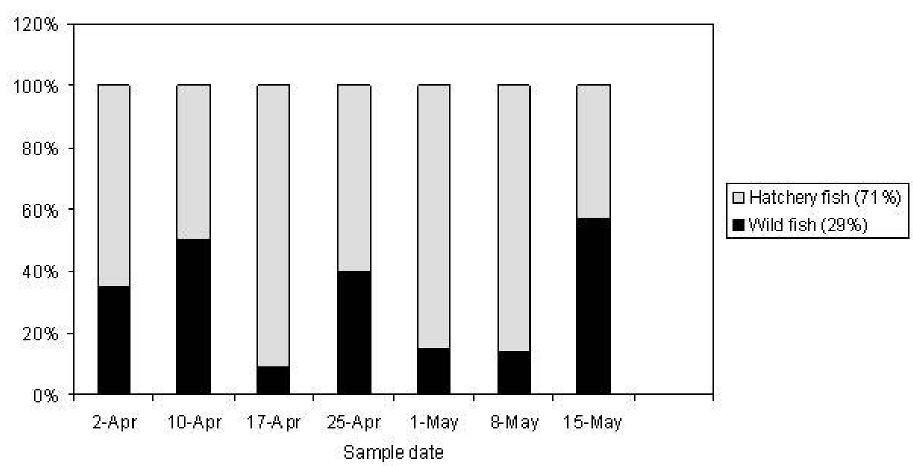


Figure 13. Choptank River adult hickory shad origin by year class for males and females combined. Data is from 2001 electrofishing survey and additional fish collected by creel survey.

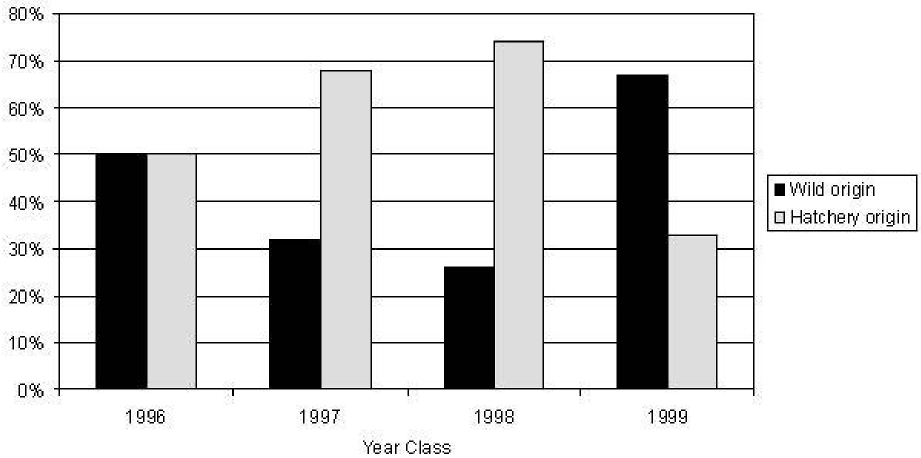


Figure 14. 2001 electrofishing and creel survey, Choptank River adult hickory shad origin by year class for females.

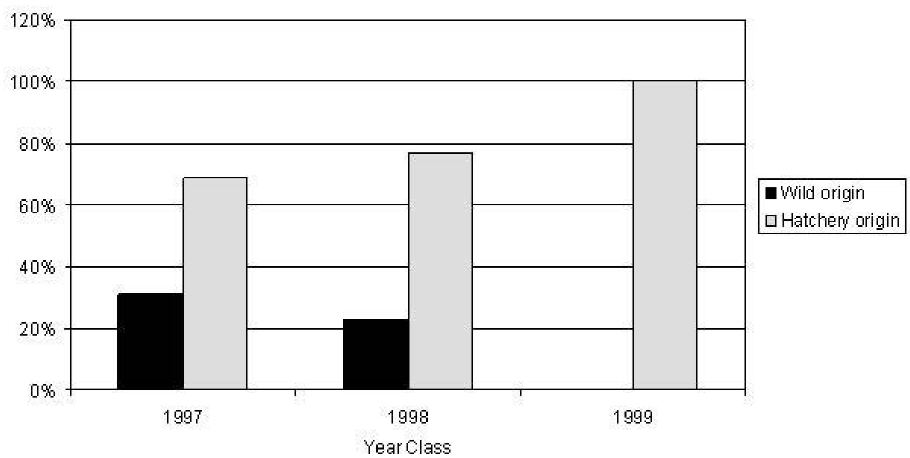


Figure 15. 2001 electrofishing and creel survey, Choptank River adult hickory shad origin by year class for males.

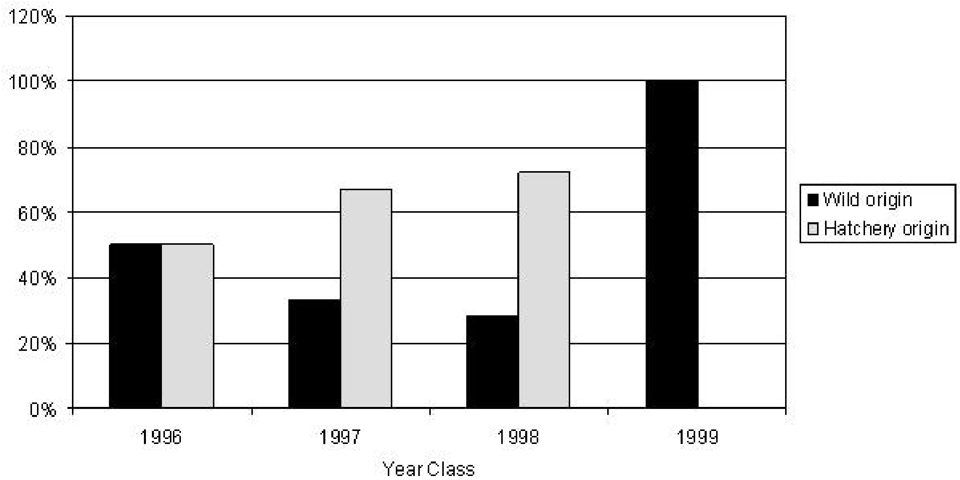


Figure 16. 2001 electrofishing survey, Patuxent River adult hickory shad origin by year class for females.

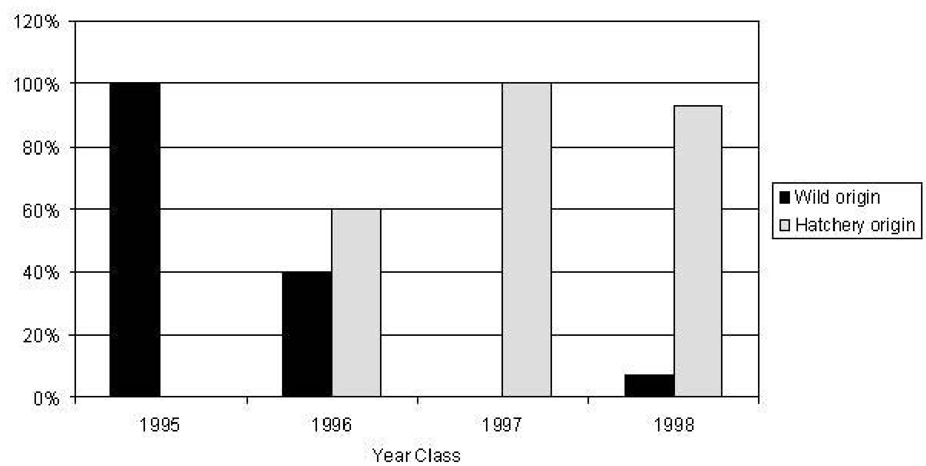


Figure 17. 2001 electrofishing survey, Patuxent River adult hickory shad origin by year class for males.

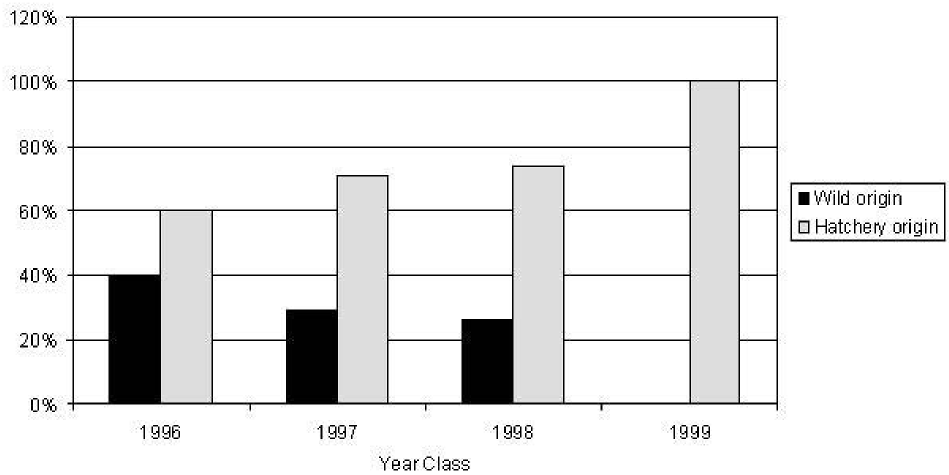


Figure 18. 2001 electrofishing survey, adult Patuxent River hickory shad origin by year class for males and females combined.

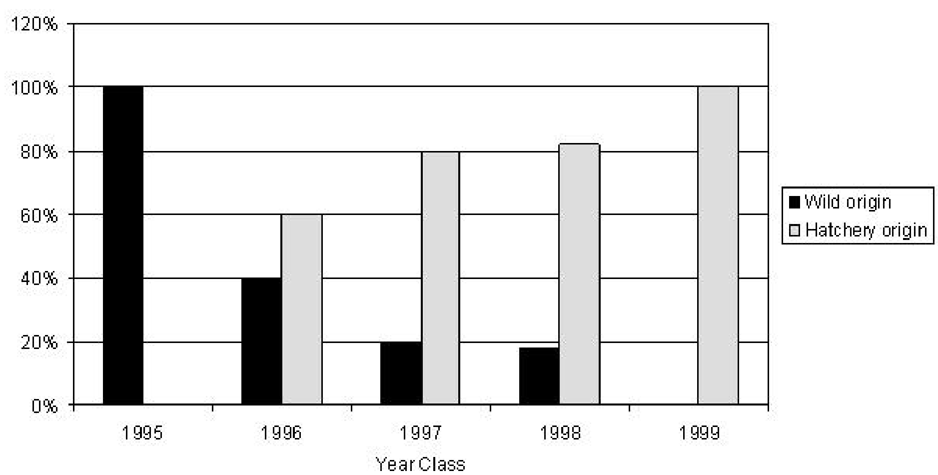


Figure 19. 2001 electrofishing survey, Patuxent River adult American shad percent composition of origin by year class, females only

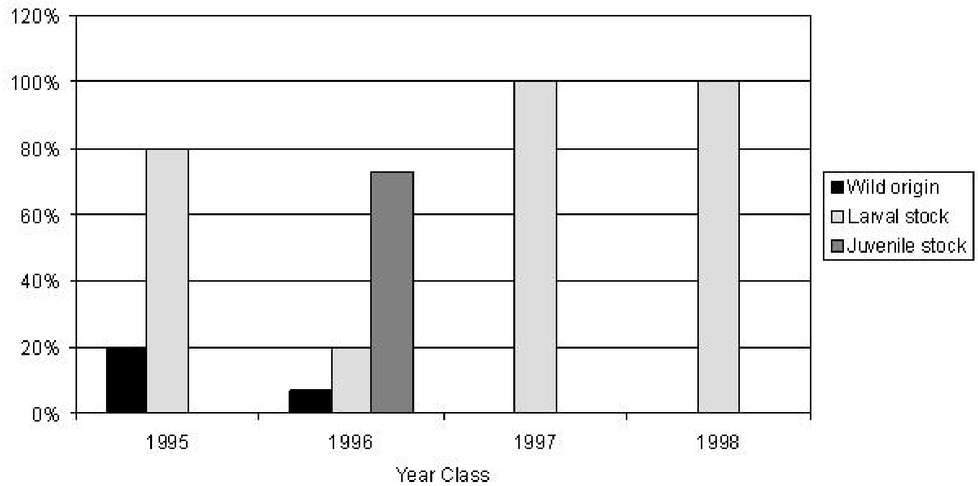


Figure 20. 2001 electrofishing survey, Patuxent River adult American shad percent composition of origin by year class, males only

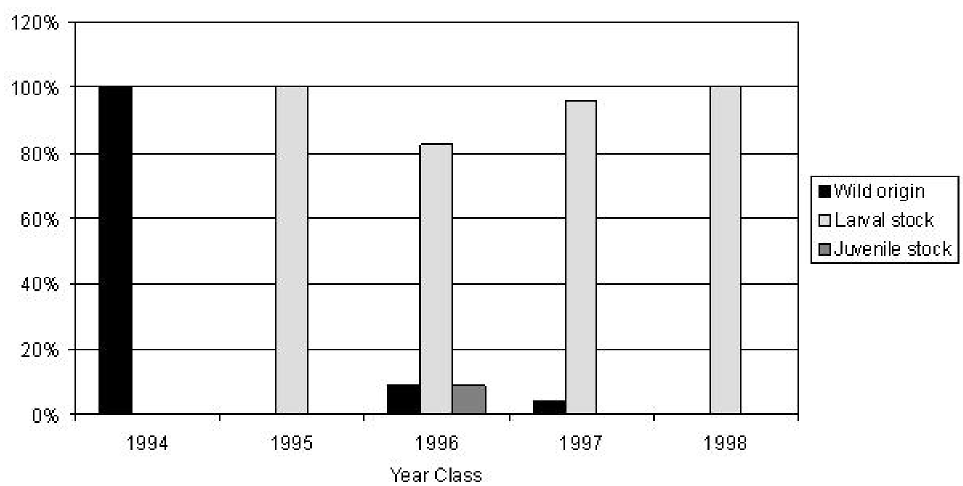


Figure 21. 2001 Patuxent River adult American shad electrofishing collections. Bars indicate proportion of hatchery fish over the sample season.

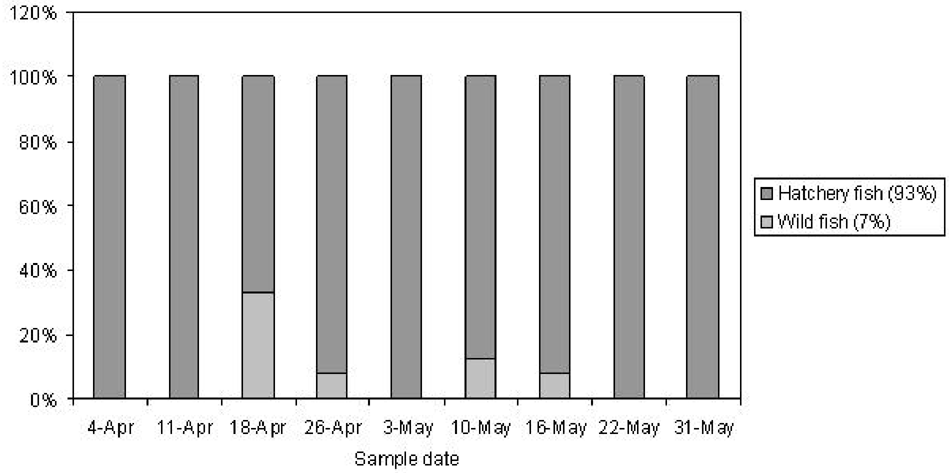


Figure 22. 2001 electrofishing survey, Patuxent River American shad adult contribution by year class and origin. Final bar represents all year classes combined, 1994-1998.

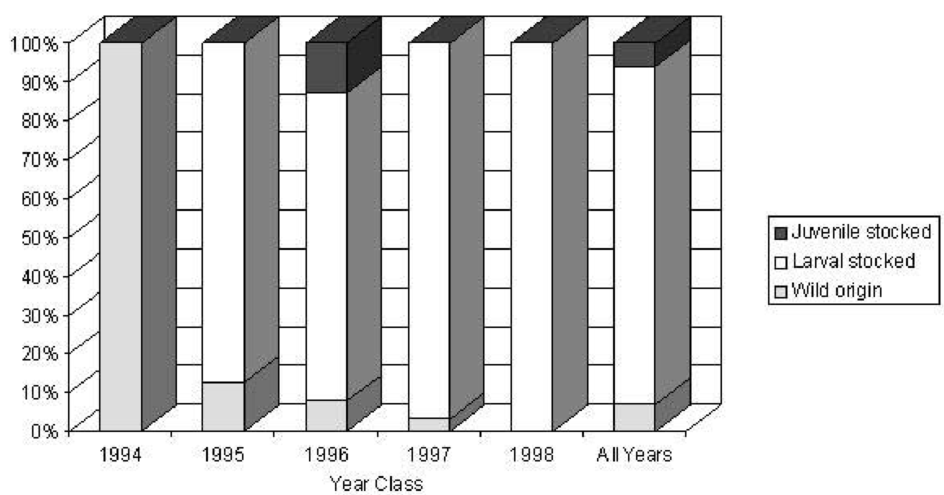


Figure 23. Susquehanna River hickory shad year class composition, sample years 1996-01. Data is from hook and line brood stock collection efforts.

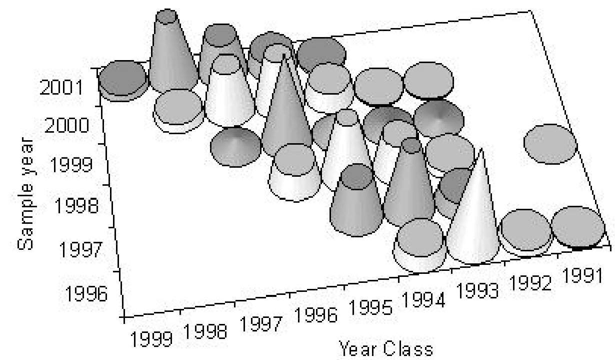


Figure 24. Susquehanna River hickory shad length frequency, Percent is the proportion of captures in each length segment.

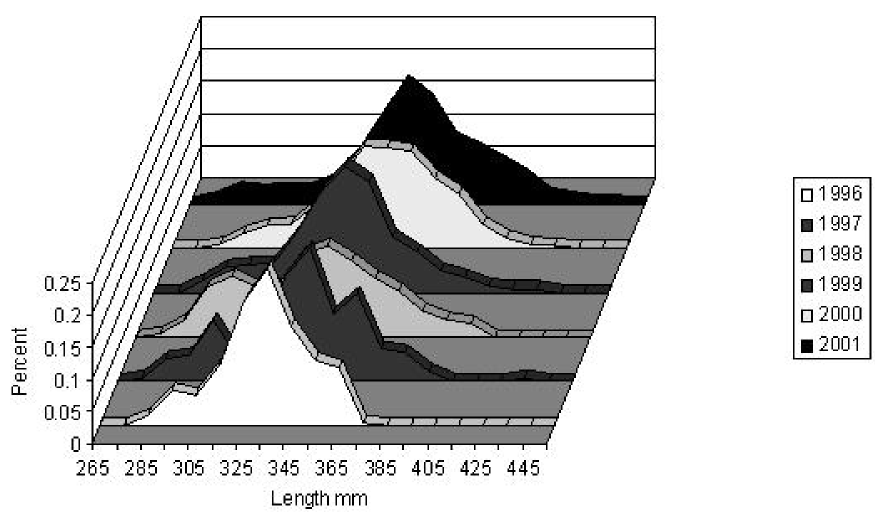


Figure 25. Hatchery component of adult American shad and hickory shad populations on spawning grounds, 1999-01. Data is from electrofishing survey in the Choptank River and Patuxent River.

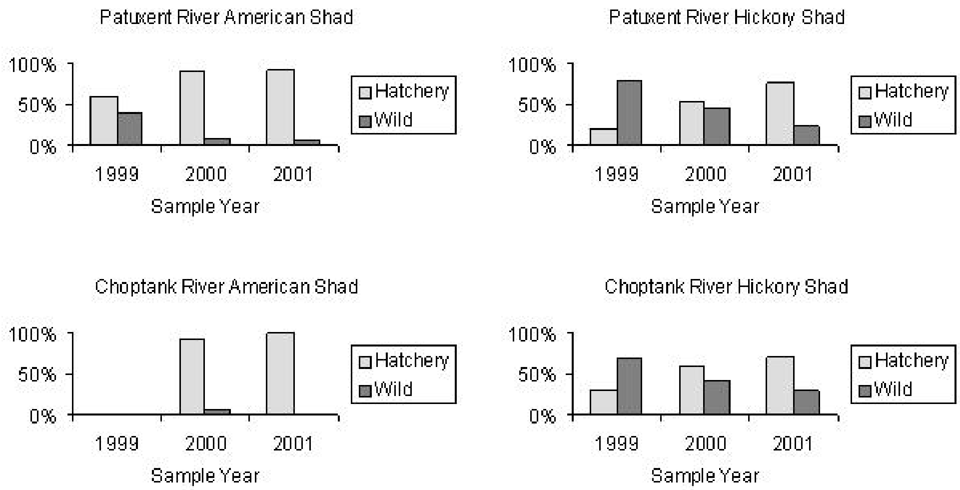


Figure 26. CPUE for hickory shad captured by boat electrofisher in 2001. CPUE is number of shad caught per minute of electrofishing time.

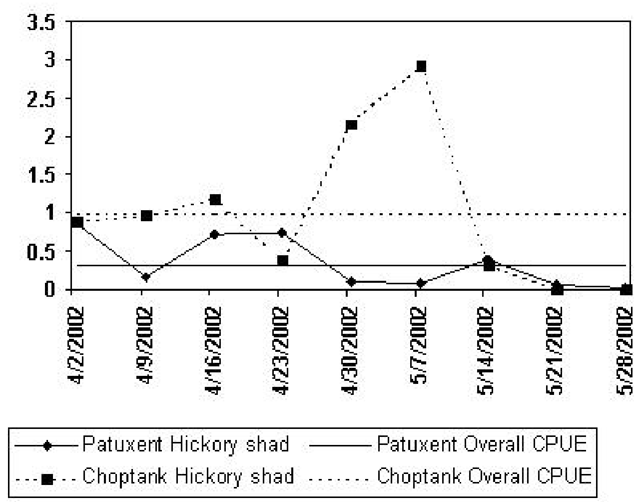
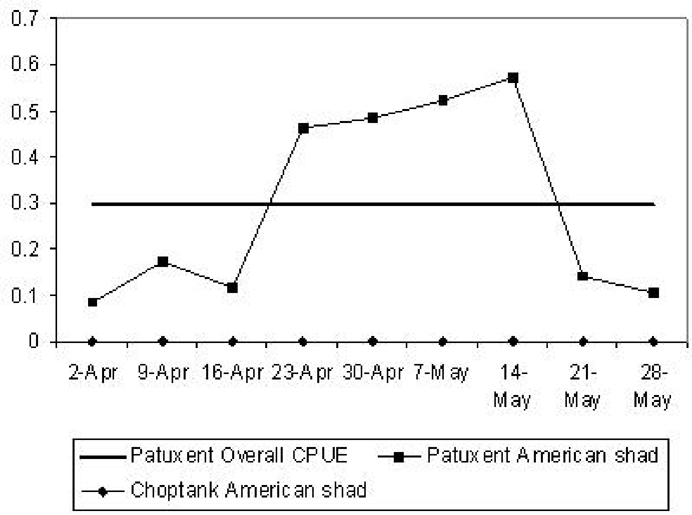


Figure 27. CPUE for American shad captured by boat electrofisher in 2001. CPUE is number of shad caught per minute of electrofishing time.



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